

Mechanistic Pathways of Probiotics in Gut Microbiota Modulation and Host Health: A Contemporary Review of Microbial, Immunological, Metabolic, Barrier-Mediated, and Pharmaceutical Translational Interactions

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ABSTRACT:

Probiotic research is now more precise and productive than it has ever been. Researchers used to speak about probiotic products in vaguer, more general terms, as 'beneficial bacteria' with a broad spectrum of positive health impacts, but now we have the knowledge to discuss probiotic strain and product effect in precise terms. One of the larger challenges in this new wave of probiotic research is the framework needed to account for a probiotic's microbial function, the delivered viable dose, the host, and even the intended clinical outcome (or endpoint). This review covers microbial research from 2020 to 2025 and combines in one place a vast array of disciplines including microbial ecology, mucosal immunology, epithelial biology, gut-brain communication, and probiotic safety and efficacy. Research goals, bibliographies, and citations were led by PubMed and MEDLINE. This review framework is called Strain-Formulation-Host-Endpoint (SFHE). With this SFHE framework, the authors describe the relationships of strain, viability, delivery form, and a host of other characteristics. This review describes many mechanisms through which probiotics exert their effects, including, but not limited to, cytokine modulation, competitive exclusion, and neuro-immune signaling. Probiotics of a particular strain/formulation appear to exert their effects most reliably in the gut. Evidence of clinically relevant effects is lacking in other areas including IBS, IBD, and psychobiotics. Consequently, the rigorous identity testing, end-of-shelf-life potency, controls of manufacture and storage, characterization of delivery systems, safety assessment, and clinically equivalent dose-response studies become all the more important in pharmaceutical translation. The SFHE model offers a viable pathway from mechanistic plausibility to the production of reproducible probiotic products and therapeutic claims.

Keywords: probiotics; gut microbiota; strain specificity; formulation; microencapsulation; quality control; immune modulation; short-chain fatty acids; intestinal barrier; gut-brain axis; pharmaceutical translation

1. Introduction

The intestinal microbiota plays an important role in metabolism by transforming nutrients, maintaining the integrity of intestinal epithelium, educating the immune system, and promoting and maintaining homeostasis throughout the body. It does this in collaboration with other microbial communities. Because of this, analyzing the effect that a microbe has on a host system by solely classified taxonomy is insufficient. Microbiome researchers are beginning to recognize the use of probiotics as interventions that are biological in nature and defined by the function of the strain of microbe being studied and the context or environment of the host that the microbe is being introduced to [1,2,3].

The distinction being made in the research is also reflected in recently published clinical guidelines. A probiotic is not equal to a single fermented food product, commensal isolate, or a single preparative synbiotic or prebiotic that contains non-viable microbes. Probiotics are live microorganisms that are administered in sufficient and concretely documented quantities, and benefits have been documented. Differentiating probiotic strains is particularly important, as they may differ in several characteristics including the strain itself, the amount that survives to reach the intestinal tract, and the ingredients and stabilizers that surround them, even if they are closely related in taxonomy at genus or species level [4,5,6,7].

A significant challenge in the probiotic literature is the disconnect between the possibility of a mechanism and the reproducibility of a probiotic product. While the mechanisms behind competitive exclusion, short-chain-fatty-acid production, immune modulation, and support of tight-junctions may be plausible, their clinical application hinges on the corresponding probiotic strain being delivered in a reproducible way, is able to withstand the manufacturing and storage processes, successfully transit to the appropriate region of the intestine, and interacts with the relevant mechanism in the target population to generate a clinically significant effect. There are reviews that discuss mechanisms of action of probiotics, but without referring to product and translational variables. These reviews treat probiotics as a homogeneous therapeutic class [5,8,9].

This review aims to achieve the following: (1) synthesize the probiotic mechanisms that are majorly microbial, immunological, metabolic, epithelial, and gut-brain; (2) further distinguish between evidence that is convergent and that which is contradictory or context dependent; (3) incorporate a mechanistic analysis lens which includes formulation, manufacturing, stability, dosage form, quality control, and safety; and (4) introduce the SFHE (Strain-Formulation-Host-Endpoint) model to probiotic biology to define products that are clinically justifiable and are reproducible from a pharmaceutical perspective.

2. Review Methodology

2.1 Review Design

This article was further developed as a critical narrative review, rather than a systematic review or a meta-analysis. The aim of this was to increase the transparency and reproducibility of the evidence selection process. This also allowed the interpretation of mechanistic, pharmaceutical, and clinical evidence of disparate study designs. The review does not capture the universe of systematic reviews, and does not intend to provide a systematic review with a pooled effect estimate, but rather, to provide a transparent critical synthesis, using specified search limits and eligibility criteria, and an evidence hierarchy.

2.2 Information Sources, Search Window, and Search Concepts

The final date for completing literature searches was set to August 9, 2026, and searches were restricted to peer-reviewed articles published from January 1, 2020, to December 31, 2025. The primary bibliographic database was PubMed/MEDLINE. Publisher or DOI directed searches, and backward or forward citation searches were performed to verify bibliographic details and identify pharmaceutically or mechanistically relevant publications. The search concepts were combined with Boolean operators, e.g., probiotic* AND

(gut microbiota OR microbiome) AND (mechanism* OR colonization OR cross-feeding OR bacteriocin*); probiotic* AND (immune OR cytokine OR regulatory T cell OR IgA); probiotic* AND (short-chain fatty acid* OR bile acid* OR tryptophan OR indole*); probiotic* AND (tight junction OR mucin OR barrier OR permeability); probiotic* AND (gut-brain axis OR psychobiotic*); and probiotic* AND (formulation OR microencapsulation OR delivery OR viability OR stability OR manufacturing OR quality control OR strain identity OR dose). Searches for the other indications were also performed for antibiotic-associated diarrhoea, irritable bowel syndrome, inflammatory bowel disease, metabolic disease, and non-alcoholic fatty liver disease.

2.3 Eligibility criteria and evidence hierarchy

Accepted evidence for the review included studies that were English language, peer-reviewed, and published primary studies, randomized or controlled clinical trials, mechanistic human studies, relevant animal or in-vitro studies, systematic reviews/meta-analyses, consensus statements, clinical guidance, and pharmaceutical development or quality-control studies published in the specified time period. Primary studies were included when they made direct associations of a specified strain or formulation with the microbiome, immune, metabolite, epithelial, clinical, formulation, or quality endpoints. Higher order studies and guidelines were included to provide context to the literature regarding consistency and safety and the clinical relevance of the study. Studies were excluded if they were published outside the date range, were published without complete or sufficient bibliographic metadata, addressed ‘probiotics’ in the literature as a generalized wellness category with no specific study or intervention, or addressed prebiotics/postbiotics and did not elaborate on the mechanism of the probiotic.

2.4 Data extraction and critical synthesis

In the analysis of the included studies, the author considered the study model or population and the strain or strain combination, as well as the dose and duration if included, the formulation and delivery, the mechanism and clinical endpoints, and the safety or product quality. The studies were categorized into the following domains: ecological, immune, metabolic, barrier, gut-brain, pharmaceutical, and clinical. Findings that were contradictory were not averaged, but instead analyzed as the SFHE suggested to see if plausible strain variability, formulation, host, or endpoint would be the reason for the findings. This was done to follow the current recommendations that probiotic claims not stray from the specificity of strain, product, and use [4,5].

3. A Strain-Formulation-Host-Endpoint Framework for Probiotic Translation

The SFHE framework, which is the main organizational structure of this review, is a more sophisticated structure for describing the interaction of the constituent elements of a probiotic. Rather than treat the taxonomic descriptor as a catch-all for probiotic potency, the SFHE framework embeds the biology that the strain represents. The formulation offers that biological capability at the viable dose in the location of intended delivery. The host offers the ecological and physiological niche where the organism operates. The endpoint describes the extent to which the effect is non-trivial and potentially valuable from a mechanistic or clinical standpoint. If there is a breach in any level, a negative result that may be encountered in a probiotic trial will still be present. The other levels of the structure may still be viable. The SFHE framework is an advancement from the other frameworks in that it establishes a more sophisticated model. The frameworks of other authors, for example, provide a level of description that accounts for the structure, the biology of the probiotic, the formulation, and the impact of the probiotic on the host [4,5,10].

Table 1. The Strain-Formulation-Host-Endpoint (SFHE) framework

Dimension	Minimum information to report	Why it changes interpretation	Typical failure if omitted
Strain	Full taxonomic name, strain designation, identity/genomic confirmation, relevant functional phenotype	Closely related strains can differ in adhesion, substrate use, antimicrobial activity, immune-facing structures, and safety.	Genus- or species-wide claims are made from strain-specific evidence.
Formulation	Dosage form, excipients/carrier, viable count, end-of-shelf-life potency, storage, GI survival/release characteristics	Manufacturing, oxygen, moisture, acid, bile, and matrix effects can change exposure before the organism reaches the target site.	The tested biological dose differs from the labeled or administered dose.
Host	Age, disease phenotype, medications/antibiotics, diet, immune status, baseline microbiota when available	Resident ecology and host physiology determine colonization resistance, cross-feeding, immune response, and responder status.	Opposite outcomes are attributed to the organism rather than host heterogeneity.
Endpoint	Mechanistic biomarker, microbiome function, clinical outcome, timing, validated assay	A change in abundance or fecal metabolite concentration is not equivalent to restored function or clinical benefit.	Surrogate improvement is overinterpreted as therapeutic efficacy.

The SFHE model accounts for the notion that transient passage may be enough in certain cases. A probiotic may not require durable colonization to exert an effect if it releases a metabolite, transiently occupies a niche, alters the local pH, and/or stimulates epithelial sensing, or provides a cross-feeding substrate during its passage through the gut. However, even a strain that is biologically active may be unsuccessful in the clinic if a formulation does not preserve the strain viability and/or if the endpoint selected does not represent a clinically relevant pathway [3,11,12].

Strain Identity, Taxonomy, and Functional Specificity

Some of the most common probiotic groups include the bifidobacteria, reclassified lactobacilli, *Saccharomyces boulardii*, *Streptococcus thermophilus*, some *Bacillus* species, and other well-characterized species that have a documented history of use, or potential therapeutic use. The taxonomy of lactobacilli is reclassifying, and as a result, the ability to identify the strain is becoming increasingly important. Clinical reports should provide the full current taxonomic name and correspond with the strain designation and, when necessary, include information regarding the culture collection and/or genomic information [4,5].

Strains may also exhibit functional heterogeneity with regard to their ability to adhere, and tolerance to acids and bile, as well as the capacity for carbohydrate metabolism, and the production of bacteriocins and extracellular polysaccharides, stress responses, cell surface structures, and immunological recognition. Factors of an industrial process may also exert selection pressures and cause other phenotypic shifts. Identity control therefore should exceed the first seed culture, and strategies for in-process control may be employed for this purpose. The phenotypic strain tracking in-process characterization by Fourier-transform infrared spectroscopy has been explored, while for multi-strain products, the use of strain-specific real-time PCR may be utilized to support the identity and stability control of the products [10,13].

5. Microbial-Ecological Mechanisms

There are a variety of conditions within the intestinal lumen, like the presence of bile acids and antimicrobial peptides, that create unique ecological niches which resident microbes occupy. Probiotic bacteria can also inhabit these niches where they can compete for available resources (e.g. mucus), alter pH, and produce antimicrobial compounds. Given the complexity of these mechanisms, a probiotic strain cannot be purely defined by the strain itself. The strain must be considered in the context of the existing ecosystem [1,3,14].

Microorganisms can also inhibit the growth of others through the binding to sites on the mucosa, production of organic acids, and the production of bacteriocins. These mechanisms require that specific concentrations be present in a particular habitat. The pH or availability of nutrients in a specific location can also influence whether a strain continues to exhibit inhibitory effects. The strongest mechanistic claims characterize an organism and a strain at a specific dose in the context of the microbial community and directed at a specific pathogen. These claims are preferred over placing generalized claims on a whole genus [4,15].

Cross-feeding is an important indirect mechanism. Lactobacilli and bifidobacteria produce lactate or acetate and produce little butyrate. Many resident anaerobes can convert lactate and acetate to butyrate and other metabolites. This can affect the energy metabolism of colonocytes and the immune tone. Thus, a probiotic might improve network function, even without persistence or engraftment. Ecology-based interpretations showed that the probiotics can even alter the microbiome function when the probiotics are not persistent [11,16,17].

Direct evidence indicates that probiotics impact strain dynamics of indigenous microbes. Analysis of paired human and animal microbiomes showed that the resident organisms changed their adaptive traits due to the probiotics, highlighting that the impact of the probiotics was not limited to simple changes in the taxonomy, and was a shift of the frameworks of the resident organisms [12]. This observation shows the limitations of interpreting the relative strain dynamics.

Immune Modulation and Mucosal Tolerance

The mucosal immune system has to be tolerant towards commensals and food antigens, while retaining rapid responses towards pathogens. There are many microbial derived molecular patterns and metabolites that can be sensed by many immune cell subtypes, which alter cytokine secretion and IgA secretion. This is described as immune modulation and is preferred to the vague ‘immune boosting’. The direction and magnitude of the response depend on the microbial ligand, host inflammatory state, tissue compartment, and dose [18,19,20].

There are pathways for probiotic signals to potentially regulate gut immune tolerance through dendritic cell conditioning and regulatory T cell support. While the activity of IL-10-secreting regulatory T cells can sometimes be advantageous, in other situations, programs that involve barrier and antimicrobial activities may be more important. Resistance to change is important in these situations because a change in a single serum cytokine is not evidence of a causative mechanism in the gut. Mechanistic studies in immunity and inflammation are greatly strengthened when cytokines and cellular phenotypes are evaluated together with mucosal immune markers, microbial communities, and clinical endpoints [18,19,21].

The immune system and the response to probiotics is dependent on the strain of probiotic and the context of the situation, as suggested by several studies. In tumor models with gut microbiome depletion caused by antibiotic therapy, the addition of the probiotic, *Lactobacillus rhamnosus* Probio-M9, was

shown to restore gut microbiota and improve the efficacy of anti-PD-1 therapy. This example illustrates that the interaction of probiotics and the immune system is dependent on antibiotic use and a specific therapy, and is not a generalized effect [22]. While these examples are helpful in understanding mechanisms, they cannot be used to justify methods that are not already established in clinical practice for cancer.

7. Metabolic Signalling: Short-Chain Fatty Acids, Bile Acids, and Tryptophan

Microbial metabolites build the link mechanism between community engagement and host activity. Acetate, propionate, and butyrate influence epithelial energy metabolism, luminal pH, immune signaling, glucose and lipid metabolism, and the enteroendocrine system. Significantly, fecal concentrations do not accurately reflect butyrate production. Stool measurements represent the net result of production, absorption, utilization, gut transit, and excretion. Higher concentrations of short-chain fatty acids in feces do not imply greater exposure of tissues to these fatty acids nor do they indicate greater activation of cellular receptors [17,23,24].

Cross-feeding provides a mechanism through which certain probiotics are able to influence short-chain fatty acid networks. Evidence from cross-feeding research indicates that an administered organism which produces lactate and acetate can enhance the activity of resident butyrate producers. Butyrate can also enhance epithelial energetics and facilitate the repair of the mucus layer while also promoting an anti-inflammatory response. This mechanism is truly a product of the community configuration and the diet of the host organism. This is due to the necessity of a partner organism in order to ferment substrates [11,16,25].

Bile acids contribute another signaling mechanism. The microbial metabolism of bile acids and their subsequent transformations can influence the modulation of the conjugated bile acid pool, which in turn alters the absorption of lipids, microbial pressure, gut motility, and the host FXR/TGR5 signaling cascade. Although these mechanisms can affect cardiometabolic and hepatic physiology, the effect of a specific probiotic will rely on its enzymes, the resident microbiome, and the bile acid physiology of the host [26,27].

Tryptophan metabolism connects diet, mucosal immunity, and neurobiology. Microbiota-derived indole metabolism affects aryl hydrocarbon receptors, epithelium, and inflammation, and is associated with tryptophan, serotonin, and kynurenine flux. The effects of these pathways make gut-brain-immune interactions highly probable, but for now, it is much harder to demonstrate direct effects on central nervous system functions, as compared to those of peripheral metabolites and preclinical studies [28,29,30].

The intestinal barrier consists of: epithelial cells; tight and adherents junctions; mucus; antimicrobial peptides; secretory IgA; immunosurveillance; the actively metabolizing microbiota; and other components. Barrier dysfunction increases exposure of both mucosal and systemic compartments to luminal antigens and microbial products. Some mechanisms of probiotics relevant to barrier integrity include modulation of the proteins of tight junctions, epithelial cell survivability, mucus, oxidative stress, and repair pathways [31,32,33].

Tight junctions demand special caution because permeability does not equal gastrointestinal symptoms. Junction proteins such as occludin; claudins; and zonula occludens; form dynamic interactions with cytokines, microbial products, and metabolic signals. Therefore, a valid barrier study must use a permeability assay of validated sensitivity and must clearly show structural evidence rather than a symptom of tight permeability [32,33].

Mucus adds another dimension of immunological and ecological input. Goblet-cell-derived mucins permit spatial differentiation of luminal microbes from the epithelium and seclude microbial niches through glycan presentation. Thus, the glycosylation and subsequent degradation of mucus can affect the

microbiota balance of symbiosis and inflammation. Therefore, the mechanistic studies evaluating the impacts of probiotics on mucus should be interpreted as changes of a complex, integrated, host-microbe environment rather than the restoration of a blank and passive mechanical layer [31,34].

9. Gut-Brain Interaction and the Psychobiotic Concept

The microbiota-gut-brain connection involves several pathways including neural, humoral, and immunological, metabolic, and macrobiotic connections. The proposed mechanisms of probiotics are via the vagal route, the modification of the hypothalamic-pituitary-adrenal (HPA) axis, inflammation and cytokine levels, the alteration of the permeability of the intestines, and various routes of signaling through short-chain fatty acids, tryptophan, and enteroendocrine cells. While these concepts are well supported by preliminary studies, the designation of a 'psychobiotic' should be maintained for a particular strain or formulation and a defined neurobehavioral outcome [29,30,35].

The translation of these concepts to humans have been hindered by small sample numbers, a mixture of strains, a variety of outcome measurement scales, and inadequate conceptual frameworks beyond peripheral measurements. For instance, decreasing self-reported stress with a measure of peripheral stress does not demonstrate a central or vagal mechanism. Psychobiotic research must have a high level of regulatory oversight for a specific formulation and a defined neurobehavioral outcome while controlling for other medications, measuring dietary restrictions, and ensuring the studies are of an adequate duration [29,30].

10. Pharmaceutical Translation: Formulation, Delivery, Stability, and Quality Control

10.1 Viability is part of the delivered dose

When dealing with live microbial products, potency and viability are one and the same. A count of colony-forming units or some other count of live organisms at the time of manufacture does not suffice when that number can be diminished during storage or transit through the gastrointestinal tract. Delivered exposure can be impacted by temperature, oxygen, moisture, water activity, compression, and excipient interactions, along with gastric acid, bile, and the choice of packaging. Therefore, a suggested probiotic dose should be reported as product-specific viable potency, and would ideally be reported with end-of-shelf-life definitions, as opposed to simply manufacturing definitions [5,9,13].

10.2 Dosage-form and delivery-system design

Standard capsules, sachets, powders, fermented matrices, and liquids all offer differing levels of protection to the organisms they contain against the stresses of processing and the gastrointestinal tract. Examples of more sophisticated protection strategies are multilayer coatings, polymeric, hydrogels, and spray dried matrices, as well as targeted intestinal delivery systems and enteric protection. These systems are far from just packaging systems; they significantly alter release location and time, as well as the biological dose of the product that is delivered to the mucosa [36].

An example of a formulation study from 2024 is that of *Bacillus clausii*. This study utilized a microencapsulation system based on maltodextrin, alginate and inulin, and focused on the appraisal of encapsulation and powder characteristics, as well as the storage stability and simulated gastrointestinal survivability, thereby providing an example of the direct connection between formulation science and probiotic viability and the idea of sustained release [37]. While studies of this nature illustrate significant potential, clinical outcomes are still predicated upon conducting a clinical study with a formulation that is matched to the study.

10.3 Manufacturing, Excipients, and Storage Stability

Manufacturing processes are an experimental stress test for biologicals. Viability and phenotype may be affected by the conditions of manufacturing and storage such as fermentation, harvesting, lyophilization

or spray drying, cryoprotectants, blending, and compression, as well as exposure to oxygen. Excipients may either protect cells or cause local hydration changes and may inflict harmful osmotic and oxidative stress to cells. Manufacturing controls are necessary to account for changes in the product prior to administration. Thus, manufacturing controls are in the causal chain of strain biology and the resulting clinical outcome [5,10].

Stability dependent on formulation has been demonstrated for non- oral products as well. In a pharmaco technical study, various gel formulations of *Lactobacillus rhamnosus* were designed, with the bacterial stability incorporated as a quality measurement along with the antimicrobial activity of the formulation, thus demonstrating the use of a probiotic organism as an active pharmaceutical ingredient [38].

10.4 Identity, Potency, Purity, and Analytical Control

Quality control must include at a minimum the assessment of identity, potency, purity, and functional stability and safety. In most cases, traditional culture-based enumeration may be insufficient to identify strains in a multi-strain preparation. Whole genome sequencing, strain-specific PCR, validated molecular enumeration, and phenotypic fingerprinting are a few alternatives. An in-process analytical study [10] showed the potential of FTIR spectroscopy for strain identity, while a separate study [13] developed a strain-specific real-time PCR method to identify and monitor the shelf-life of *Bifidobacterium longum* subsp. *longum* UAB1-14. These methods have shown that the quality of a pharmaceutical product will link the declared identity of the product to the microbial strain present in the final preparation.

10.5 Dose Standardization and Pharmacological Interpretation

Probiotic dosing cannot simply be compared to a mass dosing model for small molecules. Probiotic dosing involves consideration of viable cell numbers and cell metabolism, along with the location of cell release, time of residence, and possible interactions with diet and microbes that are currently residing in the gut. As such, more than the nominal CFU should be considered in dose-response studies. Viable delivered dose should be related to strain recovery and also to target engagement, biomarkers, clinical metrics, and adverse reactions, where applicable. The absence of such related studies can lead to the conclusion of a potentially apparent dose-response failure. In this case, such a failure may result from formulation loss, host non-response, or lack of viable biological activity [4,5].

Table 2. Pharmaceutical determinants that should accompany mechanistic probiotic claims

Development domain	Key question	Preferred evidence/reporting
Identity	Is the organism in the product the strain that was studied?	Strain designation; genomic or validated strain-specific identity method; traceable seed lot.
Potency	How many viable organisms are delivered when the product is used?	Validated viable count at release and end of shelf life; acceptance criteria; dose per unit.
Formulation	Does the dosage form protect or alter the organism?	Dosage form, excipients, encapsulation/coating, disintegration/release and GI-survival testing where relevant.
Stability	Does potency/phenotype change during storage?	Temperature, humidity, oxygen/light controls; packaging; real-time stability and strain-specific monitoring.
Manufacturing	Can the process reproducibly produce the same biological product?	Fermentation, harvest, drying, blending and batch-control parameters; contamination control.

Safety	Could the strain or product create host or ecological harm?	Genomic/phenotypic safety assessment, antimicrobial-resistance evaluation, contaminant testing, population-specific risk.
Clinical linkage	Is the marketed product equivalent to the clinically studied intervention?	Matched strain(s), dose, formulation, storage conditions, duration, indication, and endpoint.

11. Representative Primary Evidence Anchors

A contemporary review of broad scope should not rely only on reviews and guidelines. Table 3 therefore highlights representative primary studies that connect probiotic exposure to ecological, immunological, clinical, or pharmaceutical endpoints. These studies are not presented as an exhaustive evidence inventory; rather, they illustrate how mechanistic and translational claims become stronger when the intervention and endpoint are explicitly defined.

Table 3. Representative primary studies linking probiotic exposure to mechanism, clinical outcome, or product performance

Study	Design/context	Mechanistic or translational contribution	Critical limitation
[39]	Human IBS-D intervention with <i>Bifidobacterium longum</i> ES1	Linked clinical response with cytokine and zonulin measurements.	Single product/context; biomarker changes do not establish universal IBS efficacy.
[22]	Preclinical anti-PD-1 model after antibiotic disruption	Probio-M9 altered microbiota and enhanced antitumor immune response.	Preclinical oncology context; not directly generalizable to routine probiotic use.
[12]	Integrated analysis of human and mouse probiotic datasets	Showed probiotic-associated strain-level adaptive changes in resident microbiota.	Genomic association does not by itself establish clinical benefit.
[40]	Randomized double-blind placebo-controlled NAFLD study	Assessed hepatic, mucosal immune, and intestinal barrier endpoints.	Disease-specific multistrain intervention; mechanisms may not transfer to other formulations.
[41]	Randomized controlled trial in post-gestational-diabetes women	Evaluated microbiota and metabolic outcomes after probiotic exposure.	Population and baseline metabolic status constrain generalizability.
[42]	Experimental colitis model with <i>Limosilactobacillus reuteri</i>	Linked epithelial heat-shock proteins to protective effects.	Animal model; pathway requires human validation.
[43]	Randomized pediatric trial	Tested a defined multispecies formulation for prevention of antibiotic-associated diarrhoea.	Formulation- and pediatric-context specific.

[10]	Industrial process/analytical study	FTIR-based in-process phenotypic strain identity tracking.	Analytical performance must be validated for each manufacturing context.
[38]	Pharmacotechnical formulation study	Connected dosage-form composition, probiotic stability, and antibacterial performance.	Non-oral formulation; clinical efficacy not established.
[37]	Spray-dried microencapsulation study	Connected carrier composition with encapsulation, storage survival, and controlled GI release.	In-vitro/simulated GI performance does not prove therapeutic efficacy.
[13]	Analytical/finished-product study	Strain-specific qPCR for identity and shelf-life monitoring in multi-strain products.	Method is strain-specific and not a universal enumeration solution.

12. Clinical Translation by Indication

12.1 Antibiotic-associated diarrhoea

Higher levels of antibiotics in the body are associated with disruptive changes in the gut microbiome due to the loss of colonization resistance. This provides a rationale for focused fast-track studies to develop marketed products. Systematic reviews indicate potential benefit in adult and pediatric populations [44,45], and recent randomized controlled studies demonstrate that the prophylactic effects are sensitive to the formulation, the target population, their risk profile, the nature and extent of the antibiotic exposure, and the outcome of interest. Therefore, the conclusion to be drawn should not be that "probiotics prevent diarrhea." Instead, the conclusion to be drawn should be that selected strains and combinations of probiotics can provide a lower risk of diarrhea in the situations described.

Irritable bowel syndrome (IBS)

IBS can be considered a syndrome as there is phenotypic variance motivated by many mechanisms including, but not limited to, changes in gut transit, visceral and mucosal hypersensitivity, diet and microbiome changes, and immune activation. Meta-analyses of randomized controlled studies have shown that probiotics can be beneficial in IBS [46], but there is still considerable variability in strains, combinations, dose, duration, and IBS subtype. One clinical study [39] integrated barrier and immune-response measures with symptoms, providing a more focused basis for product-specific interpretation than less targeted studies.

12.3 Inflammatory bowel disease

It is possible to understand the distinction of adjunctive biology and existing treatment with the example of inflammatory bowel disease (IBD). Probiotics could alter the integrity of barriers, microbial ecology and inflammatory signals. However, the pathogenesis of IBD incorporate epithelial injuries, environmental factors, host genetics, dysregulation of the immune system and the level of activity of the disease. In clinical practice, replacing established anti-inflammatory or immunomodulatory treatment of IBD with clinically useless probiotic products is not recommended. If there is an indication of benefit from probiotics, it should be within the context of the formulation and disease and the clinical scenario [47,48].

12.4 Metabolic and liver disease

The combination of metabolic and liver diseases may be of greater biological interest, since microbiota-derived metabolites, bile acids, gut permeability and inflammatory tone may all influence insulin and hepatic-based inflammatory tone may influence insulin and hepatic inflammatory physiology.

Despite the potential, the impacts of probiotics are generally weak and inconsistent. Significant impact of probiotics will be reported based on host factors (including the baseline microbiome), diet, adiposity, and medications) and length of the clinical trial. Randomized trials of probiotics in the post gestational diabetes and non-alcoholic fatty liver disease (NAFLD) will show potential of well-defined probiotic products to influence the microbiome, metabolism and intestinal immune-barrier and mucosal endpoints [40,41]. These will also show the necessity of interpreting results on a specific basis for disease and product.

12.5 Gut-brain and Other Systemic Applications

While pursuing psychobiotic, allergic, and dermatological studies, among other systemic applications, research is more active. Mechanistic studies may be more difficult to implement. The further an endpoint is from the GI tract, the more likely there needs to be justification in terms of intermediate target engagement studies rather than shifting focus to endpoint symptomology. In the case of gut-brain studies, this requires the integration of product characterization with the measurement of metabolites, immune, endocrine and autonomic systems, or other variables which are interpretable mechanistically. Statements pertaining to the use of any probiotic in the treatment of anxiety, depression, cognitive disorders, etc. will be unable to be substantiated [29,30,35].

Table 4. Clinical evidence interpreted through the SFHE framework

Indication	Overall evidence pattern	Most important SFHE modifiers	Appropriate interpretation
Antibiotic-associated diarrhoea	Relatively coherent preventive signal for selected products in defined settings.	Strain/formulation, antibiotic regimen, baseline risk, age, diarrhoea definition.	Use product- and population-specific evidence; avoid class-wide claims.
Irritable bowel syndrome	Heterogeneous; some symptom improvement in meta-analyses and individual trials.	IBS subtype, diet, baseline microbiota, strain combination, duration, endpoint.	Promising but not a universal probiotic indication.
Inflammatory bowel disease	Mechanistically plausible but clinically variable; adjunctive roles may be narrow.	Disease type/activity, concomitant therapy, formulation, outcome definition.	Do not substitute for established therapy; require scenario-specific evidence.
Metabolic/NAFLD	Modest and inconsistent changes across metabolic, microbiome, barrier, and inflammatory endpoints.	Diet, adiposity, medications, baseline microbiota, duration, viable dose.	Interpret as product- and phenotype-specific rather than disease-class efficacy.
Psychobiotic applications	Mechanistic rationale strong; human clinical translation uneven.	Strain, psychiatric phenotype, medication, outcome mechanistic biomarker, diet, scale,	Avoid generalized mental-health treatment claims without robust strain-specific trials.

13. Contradictory Evidence and Boundary Conditions

In probiotic literature, multiple contradictions have been documented. First, the functional effect may still occur even if colonization is absent. This is the case because the impact of probiotic passage may be short-lived, but is still enough to release metabolites or engage with receptors. Second, there may be increases in 'beneficial' taxa, but functional enhancements may still be absent. This is because there is little connection between the presence of taxa and their metabolic output. Third, fecal metabolites may actually be expressed in a manner that is unrelated to their exposure in the host. This is because there may be variances in absorption and utilization that affect the concentration of metabolites in the feces. For these reasons, a pathway-level interpretation will always be superior to a taxonomic change [1,11,23].

A separate set of contradictions is seen with the immune context. Microbial signals can participate in an immune-reactive context and also, in another, promote immune tolerance. The same is true of cytokines, which can appear beneficial in one modeling of inflammation but be desirable in another. The SFHE model solves this problem by specifying the host's inflammatory phenotype and endpoint before directing the model to be 'anti-inflammatory' or 'immune-enhancing' [18,19].

The third source of inconsistency is pharmaceutical, as opposed to biological. Two studies may report the same species while giving different strains, doses, excipients, shelf lives, or release profiles. If the actual exposure to the intestines is different, inconsistent clinical outcomes will follow. Therefore, shelf-life end-testing, identity testing, and formulation testing should be considered scientific variables, not manufacturing variables [5,10,13].

Safety, Product Quality, and Regulatory Translation

Generally, established probiotic strains have good safety profiles in healthy populations. However, safety is not guaranteed, and potential issues include bacteremia or fungemia in susceptible hosts, contamination, excessive immune responses, unwanted metabolism, antimicrobial resistance and virulence. Sick, immunocompromised, catheterized, premature, and otherwise vulnerable patients need special care. Safety evaluation should be strain-, product-, dose-, and population-specific [5,9].

From the U.S. Pharmacopeia point of view, safety evaluation of probiotics is unlike typical chemical toxicology because live organisms can replicate and behave differently when altering host environments. Modern safety evaluation may include a history of safe use, taxonomic and genomic commitments, antimicrobial and purging manufacturing safety, and clinical safety of the intended population [9,49].

Product quality matters as well. A label that only specifies species and provides a CFU count at the time of manufacturing does not guarantee that the clinically studied strain is actually present at the claimed level of viability. Therefore, the ability to perform strain-specific analyses, real-time stability and contamination assays, as well as implement controls to ensure the integrity of the packaging and traceability of the manufacturing process, is critical for reproducibility. These factors become even more important as the products progress from foods or supplements to live biotherapeutics or products that make disease-treatment claims [5,10,13].

15. Limitations of the Current Evidence Base

There is a limited evidence base because of heterogeneity in strain identity, multistrain combinations, dose, formulation, storage conditions, diet and antibiotic exposure, baseline microbiota, population phenotype, endpoint definitions, follow-up periods, and adverse-event reporting. Numerous studies focus on the measurement of symptoms and fail to address strain-level measurement, metabolomics, mucosal immunology, or validated measurements of barrier function. Additionally, many of the studies that describe mechanisms do not tackle clinically relevant endpoints. This presents a clear break in the chain of

causation for the use of the product and the resulting engagement of the target and benefit to the patient [5,48].

Despite what has been identified, there is still a risk of overestimating how robust the relations between microbiomes and health outcomes are. For one, describing a genus as part of a healthy microbiome does not mean its addition will recreate that healthy microbiome. A healthy microbiome can be characterized by the presence of a genus deemed 'healthy.' Future work should emphasize functional measurements instead of the relative measurements of genus abundance such as strain persistence, metabolic flux, and pathway activation, and validated host biomarkers, and clinical measurements [1,12].

There are some limitations regarding methodology in this review. The review is a structured critical narrative review, not a systematic review. Therefore, it is not exhaustive in reporting database searches, scoring for bias is not formally reported, and there are no effect size estimates. Its aim is to enhance the translation from the mechanistic to the pharmaceutical. The limitations should be kept in mind while examining the proposed framework. The aim of the framework is to act as a reporting and reasoning framework and not a validated clinical decision framework.

16. Research and Development Priorities

In advancing studies with probiotics, there should be comprehensive intervention delineation and specification for the following: current strain name and designation, and identity method. Along with that, indicate viable dose, dosage form, excipients, storage conditions, end-of shelf-life potency. Specify the target host phenotype and the concomitant therapy with antibiotics or other influential pharmaceuticals, diet, and baseline microbiota to allow interpretation of response variability. Designated clinical outcomes should be proposed and mechanistic measurements should be linked to clinical outcomes as a means to test a specific causal pathway [4,5].

Pharmaceutical research and development should strive to combine functions with the viability of a product in addition to conducting development with quality-by-design in mind. In vitro studies should go beyond assessing the organism's survival in simulated gastric fluid. In addition to that, investigators should pose the following questions: Does the manufacturing process preserve the organism's functional phenotype and identity? Does the dosage form release the organism in the intended location? Is the potency consistent throughout the shelf-life of the dosage form? Does the enhancement of the organism's delivery system improve the engagement with the therapeutic target in humans? The new developments of delivery systems and encapsulation methods, as well as polymeric systems and advanced targeted delivery systems, make such questions more answerable [36,37].

Mechanistic clinical studies should combine multiple omics, but this should not be done in an arbitrary manner. Metagenomics should be used to verify the changes at the level of community and of the strain, metabolomics should be used to measure the levels of short chain fatty acids, bile acids, indoles, and other metabolites, and modulation of transcriptomic and proteomic measurements should be used in the assessment of epithelial and immune responses. These technologies should be used to make the connection between the product in question and a therapeutic target of a biological pathway and the clinical outcome, rather than to produce a plethora of dimensions and associations for which no causal structure can be defined [1,50].

Lastly, analysis of responders should be done in advance. Parameters such as the baseline microbiota, diet, host genetics, age, disease phenotype, and the presence of other medications can all alter the effect of probiotics. Therefore, the SFHE framework should serve as an example for conducting research, as it requires researchers to clarify the product delivered, the biological strain, the host context, and the endpoint before drawing conclusions on effectiveness.

17. Conclusion

Current microbiological research does not substantiate genus-based, species-based, or product-based therapeutic claims for probiotics. Probiotics may compete, cross-feed, and signal. They may also modulate the immune system, aid epithelial tissue and substrate, and facilitate gut-brain signaling. However, competition, cross-feeding, and signaling may only be relevant when connected to a strain, a consistent formulation and dose that survive the gut, host, and endpoint. SFHE helps connect mechanism to pharmacology.

The modified evidence base demonstrates that probiotic research must integrate pharmaceutical science. Identity, quality, and safety, as well as end-of-shelf-life determination and controlled gastrointestinal delivery, are not secondary product issues. They determine whether a mechanism of action may be applicable or repeatable. The most promising research in the field of probiotics will focus on strategies that integrate ecologic, immunologic, and pharmacology research and apply them within a rigorous clinical framework in order to provide claims that are population and context specific.

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